OFFICE OF NAVAL RESEARCH

END-OF-THE-YEAR REPORT

PUBLICATIONS/PATENTS/PRESENTATIONS/HONORS/STUDENTS REPORT

For

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Studies of Monolayers of Cyclodextrin and Flavin Derivatives

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PR Number: 97PR05781-00
Contract/Grant Number: N00014-97-1-0665
Contract/Grant Title: Studies of Monolayers of Cyclodextrins and Flavin Derivatives
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a. Number of papers submitted to refereed journals, but not published: __2__
b. + Number of papers published in refereed journals (for each, provide a complete citation): __0_
c. + Number of books or chapters submitted, but not yet published: ___0_
d. + Number of books or chapters published (for each, provide a complete citation): __0__
e. + Number of printed technical reports/non-refereed papers (for each, provide a complete citation): _0__
f. Number of patents filed: __0__
g. + Number of patents granted (for each, provide a complete citation): __0__
h. + Number of invited presentations (for each, provide a complete citation): _1___
i. + Number of submitted presentations (for each, provide a complete citation): __3__
j. + Honors/Awards/Prizes for contract/grant employees (list attached): __0__
(This might include Scientific Society Awards/Offices, Selection as Editors, Promotions, Faculty Awards/Offices, etc.)
k. Total number of Full-time equivalent Graduate Students and Post-Doctoral associates supported during this period, under this PR number: __1__
Graduate Students: _1___
Post-Doctoral Associates: _0___
including the number of,
Female Graduate Students: __1__
Female Post-Doctoral Associates: __0__
the number of
Minority* Graduate Students: __1__
Minority* Post-Doctoral Associates: __0__
and, the number of
Asian Graduate Students: __0__
Asian Post-Doctoral Associates: __0__
l. + Other funding (list agency, grant title, amount received this year, total amount, period of performance and a brief statement regarding the relationship of that research to your ONR grant)

+ Use the letter and an appropriate title as a heading for your list, e.g.: b. Published Papers in Refereed Journals, or, d. Books and Chapters published. Also submit the citation lists as ASCII files via email or via PC-compatible floppy disks
* Minorities include Blacks, Aleuts, AmIndians, Hispanics, etc. NB: Asians are not considered an under-represented or minority group in science and engineering.

Part I. (continued)

H. Invited Presentations:

Khan, A. R.; Forgo, P.; Tain, S.; D'Souza, V. T. "Selective Modifications of Cyclodextrins", 9th International Symposium on Cyclodextrins, Santiago de Compostela, Spain, May 31, 1998.

I. Submitted Presentations:

Stine, K. J.; Khan, A. R.; D'Souza, V. T. "Synthesis of 10-Alkyl-Isoalloxazines for Monolayer Studies", 32nd Midwest Regional Meeting of the American Chemical Society, Lake of the Ozarks, MO, October 30, 1997.

Stine, K. J.; Andrauskas, D. M.; Khan, A. R.; D'Souza, V. T.; Friedman,. R. M.; Liu, J. "Self-Assembled Monolayers of Modified Flavins", 32nd Midwest Regional Meeting of the American Chemical Society, Lake of the Ozarks, MO, October 31, 1997.

Andrauskas, D. M.; Khan, A. R.; D'Souza, V. T.; Stine, K. J. "Self-Assembled Monolayers of Synthetic Flavins on Gold", 72nd Colloid and Surface Science Symposium, Penn State University, June 22, 1998.

L. Other funding:

"Spectroscopic Studies of Monolayers of Cyclodextrin Derivatives", UM-St. Louis Research Award, $7250 funded, 12/96-12/97.

This grant was used to purchase a refurbished krypton ion laser for studying self-assembled monolayers of modified cyclodextrins.

Part II.

a. Principal Investigator: Dr. Keith J. Stine

b. Current Telephone Number: (314) 516-5346

c. Cognizant ONR Program Officer: Dr. Harold E. Guard

d. Program Objective

The goal of the research program is to develop the surface chemistry applications of cyclodextrins and flavins in a selected number of areas and is a combined effort of organic synthesis and physical measurements. A principle goal is to form self-assembled monolayers (SAMs) of sulfide modified cyclodextrins and evaluate their use in modifying substrates for surface-enhanced Raman spectroscopy in order to improve the sensitivity and selectivity of SERS for the application of environmental sensing. Another goal being pursued is to form SAMs of immobilized artificial flavoenzymes, consisting of a flavin group on the secondary side of the cyclodextrin cavity and methylsulfides on the primary side, and to evaluate the electrochemical and electrocatalytic behavior of these films. In relation to this goal, SAMs of flavin-sulfide derivatives are being prepared and their structure and electrochemical behavior studied. Amphiphilic flavins are being studied in mixed micelles and incorporated into Langmuir-Blodgett films.

e. Significant Results 97/98

During the past year, the following significant results have been achieved:

(1) The synthesis of flavin derivatives for SAM formation has been continued. These flavins are modified in the N-10 position with a methylthiopropyl group or other alkyl groups and can have electron-withdrawing groups such as trifluoromethyl at the 7- position. It is possible to form SAMs of flavins with varied reduction potential; in particular, the reduction potentials for the 7-carboxyl (-0.36 V) and 7-trifluoromethyl (-0.25 V) vs. Ag|AgCl show the versatility of this approach. Electron transfer rate constants were measured using scan rate dependent cyclic voltammetry. The films were studied by x-ray photoelectron spectroscopy and their domain structure imaged using low-voltage field emission SEM at Monsanto. The films have been found to catalyze the oxidation of NADH to NAD^+.

(2) The Raman spectroscopy apparatus is nearly established. Problems with stray light rejection (Rayleigh scattering) should be overcome by the recently purchased super-notch plus filter from Kaiser Optical Systems. The regular grade notch filter was found inadequate for this purpose. The methods for polishing silver foil and electrochemical roughening have been investigated and practiced by the PI.

(3) An automated surface tensiometer has been acquired on loan from Mallinckrodt for studies of mixed micelle cmc values needed for the flavin catalysis studies. Graduate student ShaDonna Shaffer has been trained by a Mallinckrodt colleague and is now using the instrument.

(4) Extensive 500 MHz NMR data have been acquired for the previously synthesized compounds.

f. Summary of plans for the next year's work

In the following year, efforts will focus on:

(1) The formation of self-assembled monolayers of the available sulfide derivatives of α-, β-, and γ- cyclodextrin on roughened silver or gold electrodes and their study using SERS. It is hoped that the surface modification will lead to SERS surface with selectivity to the favored guests of the cyclodextrins. Guest molecules studied will include some chlorinated and polychlorinated biphenyls as examples of important pollutant molecules.

(2) The investigation of the electrochemical behavior of self-assembled monolayers and Langmuir-Blodgett films of flavins with their reduction potential modified by electron-withdrawing groups. In addition, study the catalytic properties of the SAMs of the cyclodextrin-flavin derivatives. The methylsulfide cyclodextrin-flavin conjugate needs to be resynthesized to achieve a higher degree of substitution. It is hoped that these films may be useful for the catalytic reduction of oxygen and the oxidative regeneration of nicotinamide cofactors (NADH → NAD^+). The lateral microstructure of the Langmuir-Blodgett films on an ITO electrode surface will be imaged using fluorescence microscopy taking advantage of the natural fluorescence of flavins.

(3) The investigation of the catalytic properties of the flavins modified with alkyl chains in mixed micellar media, with the goal of adjusting (increasing) their reduction potential to increase their oxidizing capacity. The substrates studied will be synthetic nicotinamides, and the natural substrate nicotinamide adenine dinucleotide (NADH). A partially fluorinated cationic surfactant will be synthesized with the goal of increasing the catalytic rate by exploiting the affinity of fluorocarbons for oxygen, required to reoxidize flavin from the reduced form.

(4) Langmuir film studies will be undertaken on mixed films of an amphiphilic cyclodextrin with some phospholipids known to form tubular structures in solution.

g. Names of Graduate Students and Postdoctorals currently working on the project:

Graduate Students:

Ms. ShaDonna Shaffer (on ONR support)
Mr. Linyong Mao (on departmental support)
Ms. Donna M. Andrauskas (part-time student, self-supporting)

Part III

Explanatory text for viewgraph 1 (introductory viewgraph)

Our research efforts center around the development of surface films (SAMs, LB films) and aggregates of derivatized cyclodextrins and flavins. Cyclodextrins are cyclic oligosaccharides of glucose capable of forming inclusion complexes with many organic species and are widely applied in agriculture, pharmaceutics, and analytical chemistry. The flavins studied are synthetic versions of the coenzymes FAD or FMN utilized by the large family of flavoenzymes. Nicotinamides, also coenzymes, are natural substrates for flavins in their reduced form. Applications of cyclodextrin surface chemistry are envisioned in the area of chemical sensors and controlled release. Application of flavins in films and aggregates are envisioned in the area of biomimetic catalysis.

Explanatory text for viewgraph 2

A flavin derivative possessing a methylthiopropyl group on the N-10 position and a carboxyl group on the 7- position has been studied as SAMs on gold. The films have been found to exhibit catalytic activity for the oxidation of the coenzyme NADH to NAD^+. The flavin group exhibits fast, quasi-reversible electron transfer. Angle-resolved XPS data suggest that the carboxyl group may be oriented away from the interface and that the molecules are physisorbed with the methylsulfide having little preference in its orientation. A low-voltage field emission secondary electron microscope image shows small (~ 100 nm) connected flavin domains incommensurate with the grain structure of the underlying gold film.

Explanatory text for viewgraph 3

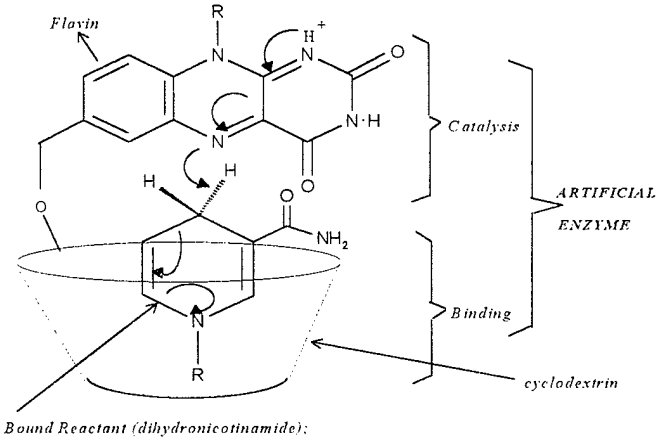
A per-6-methylsulfide derivative of β-cyclodextrin functionalized on the secondary side with an anthraquinone unit has been prepared and found to form SAMs on gold of coverage ~80%. SAMs of cyclodextrins with electroactive groups covalently attached to the secondary side can be used to study the effect of the presence of a guest molecule on the electron transfer from the group to the electrode. The cyclic voltammograms shown here were measured in a pH 10 phosphate buffer in the absence and presence of the guest molecule chlorobenzene at 260 mV/sec scan rate and there appears to be a shift of the peaks suggesting less efficient electron transfer in the presence of the guest molecule. The electrochemical behavior of the anthraquinone is pH dependent, with well-resolved reduction and oxidation peaks seen only for basic pH values above ~6.

STUDIES OF MONOLAYERS OF CYCLODEXTRIN AND FLAVIN DERIVATIVES

Keith J. Stine, University of Missouri

Objectives:

- Synthesize amphiphilic cyclodextrins and flavins for interfacial binding and catalysis.
- Evaluate host-guest complex formation by the monolayers, chemical sensors being the ultimate application.
- Evaluate electrocatalytic properties of immobilized flavins and flavocyclodextrins using their homogeneous catalytic activity as a guide.



Approach:

- Synthesize modified cyclodextrin and flavins: amphiphiles, sulfides, flavocyclodextrins.
- Evaluate monolayer properties using techniques including electrochemistry, surface plasmon, surface Raman, Langmuir-Blodgett film study.
- Evaluate the oxidation capabilities of the flavin amphiphiles and immobilized flavins.

Accomplishments:

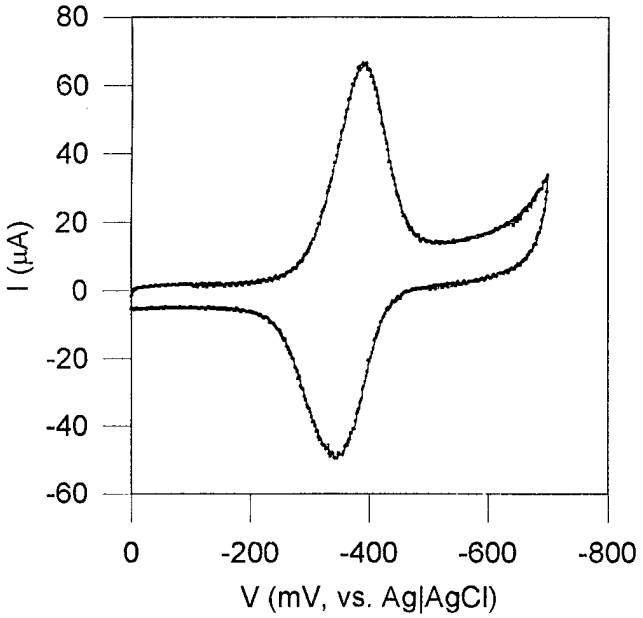
- Developed synthetic routes to modified cyclodextrins and flavins.
- Characterized interfacial binding by Langmuir-Blodgett film of cyclodextrin amphiphile
- Characterized electron transfer in SAM of flavin sulfide derivative and demonstrated oxidation of NADH by the SAM.

Transitions:

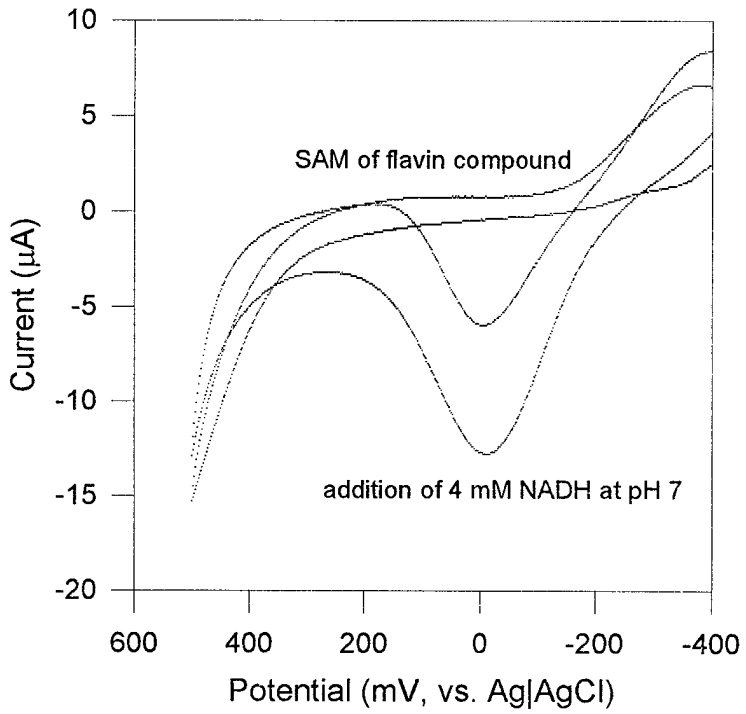
- Established collaboration with Monsanto to characterize self-assembled monolayers using XPS and low voltage field emission secondary electron microscopy.
- Established collaboration with Mallinckrodt Medical to characterize the amphiphilic derivatives using light scattering and surface tensiometry.

2. Self-Assembled Monolayers of Flavin-Sulfide Compounds

Cyclic Voltammogram of flavin SAM on gold electrode.



Oxidation of NADH by Flavin SAM



3. Self-Assembled Monolayers of a Cyclodextrin-Anthraquinone Derivative

Cyclic Voltammograms of Methylsulfide-cyclodextrin-anthraquinone